analyzed correlation between ADP-ribosyltransferase activity and the adjuvant activity of a recombinant of *E. coli* heat-labile toxin mutant. Based on the research result, it has been proposed that the two activities are closely related to each other and are not separable from each other (N. T. Lycke et al., Eur. J. Immunol. 22, 2277-2281, 1992). Other reports also note that recombinant mutant toxins with reduced enzyme activity were accompanied with decreases in adjuvant activity (for example, J. D. Clements et al., Vaccine 6, 269-277, 1988, test sample; *E. coli* heat-labile toxin; W. J. Black et al., Science 240 (4852), 656-659, 1988, test sample; *Bordetella pertussis* toxin).

In other words, in previous theories, it has been believed that a high level of reduction of toxin activity does not lead to development of adjuvants with high safety, because the reduction is associated with the decrease of the immuno-enhancing activity. And yet the attenuated toxin of the present invention demonstrates that the activity of enhancing immunity is substantially the same as that of the natural one, in some cases even when the toxic activity is reduced to at least one-two thousandth,, more preferably at least one-ten thousandth, as compared with that of the natural one. These are novel findings first revealed based on the studies for long years by the present inventors.

It remains to be clarified why toxic activity and activity of enhancing the immunity are separable in the attenuated toxin. However, a similar phenomenon has been found for a variety of vaccines using toxoid as the antigen. These attenuated toxins have been known to retain immunogenicity. An explanation can be offered based on the mechanism. For example, the following explanation can be given for the adjuvant activity of the attenuated cholera toxin. Since the expression of toxic activity (for example, ADP-ribosyltransferase) requires a specific three-dimensional structure formed by a appropriately large-sized oligopeptide, even a minor change of three-dimensional structure results in loss of the activity. On the other hand, the activity of enhancing immunity can be expressed by the whole toxin molecule or by a smaller peptide thereof, and the activity might be maintained with minor changes in the primary structure

of the toxin protein.

It has been known that cholera toxin evokes various immuno-enhancing reactions when acting as an adjuvant. For example, (1) it enhances the permeability of antigen. It binds to a receptor and activates helper T cells. It stimulates the proliferation of type-1 and type-2 helper T cells. Further, (2) it enhances the production of a variety of cytokines, for example, interferon-gamma, and interleukins 1, 4, 5, 12, etc. There is a possibility that in these immune responses, the toxin molecule effects without any structural changes thereof in the case of (1), and the toxin molecule digested to peptide fragments show the effects in the case of (2). However, details of this remain to be resolved.

An antigen protein is processed to peptide fragments in series by the action of proteosomes. The resulting peptides are presented by antigen-presenting cell. The presentation stimulates the subsequent series of immunoreactions: for example, the activation and proliferation of helper T cells, the production of a variety of cytokines, antibody production by B cells, and the like. There is a possibility that a similar mechanism to that described above works for the expression of adjuvant effects of toxin.

The above-described possibility is a hypothesis at present. While the toxic activity is mainly due to a single mechanism, the adjuvant activity is exhibited by multiple mechanisms; therefore, it can be presumed that the attenuated toxin still retains the activity of enhancing immunity even after losing the toxin activity.

Toxin:

Attenuated toxins which constitute the adjuvant of the present invention are derived from natural toxins produced by an organism such as bacterium, fungus, plant or animal. A bacterial toxin is a particularly preferred toxin to be used as the adjuvant of the present invention. It is easy to produce bacterial toxin on a large scale and thus economically advantageous. Further, toxins derived from bacteria include, but are not limited to, toxins that have excellent adjuvant activity, e.g., cholera toxin. These include single proteins and conjugated proteins. Specifically, in the context of the present invention, the term "toxin" refers to a proteinous substance having

toxic activity, which is produced by an organism in nature or a fragment or a subunit thereof. However, in the context of the present invention, the term "toxin" does not include low molecular toxins with a molecular weight less than or equal to 500 with no expected adjuvant activity. Further, toxin as defined in the present invention comprises neither toxic substances (such as poisons), which are comprised in a part of compounds synthesized by organic chemistry, nor toxic heavy metals.

Illustrative examples of natural toxins contemplated by the present invention include but are not limited to the following:

1. Bacterial toxins, including but not limited to, cholera toxin, diphtheria toxin, pertussis toxin, staphylococcus α toxin, staphylococcus β toxin, *Vibrio parahaemolyticus* thermostable hemolytic toxin, heat-labile toxin of pathogenic *E. coli*, Shiga toxin, pyocyanic enterotoxin A, etc.;
2. Fungus toxins, including but not limited to, canditoxin, fumigatoxin, toxins of mushrooms, etc.;
3. Plant toxins, including but not limited to, ricin, etc.; and
4. Animal toxins, including but not limited to, snake venom, bee toxin, toxins from Arthropoda (for example, scorpion venom), etc.

Like pigments, antibiotics, and such, toxins are so-called "secondary metabolites". Original physiological roles of toxins in toxin-producing microorganisms, plants and animals often remain unclear. Toxin production, as a characteristic nature of secondary metabolite production, shows strain-specificity (i.e., is specific to each individual in plant and animal) and not all organisms belonging to a species are capable of producing the toxin. The frequency among strains of the same species to produce the same toxin is not constant and depends on the toxin. The same toxin can be produced by a sub-species or a related species. Multi-component toxins, with toxins having partially altered structure to a small degree, are often produced by wild-type strains, related strains and artificially mutated strains.

Thus, a mixture of multiple toxins can be used as the adjuvant of the present invention. Further, when a toxin molecule consists of multiple subunits, one or more subunits that are required for the adjuvant activity can be selected and used as the adjuvant of the